# MEMBRANE LIPID ORDER IN NORMAL AND CATARACTOUS HUMAN LENSES

by

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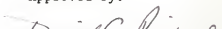

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SECTION 1

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

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I dedicate this thesis to my children, Tonya and Tate. I promise I'll make it to every ballgame next summer.

# SECTION II

# LITERATURE REVIEW

# LITERATURE REVIEW

## INTRODUCTION

The human lens is a unique biological structure with an unusually high protein content, at least 35% on a wet weight basis, yet it remains transparent (36). During senile cataractogenesis the lens becomes opaque and loses its refractory ability. The events leading to this condition remain obscure.

Like the red blood cell, the lens fiber cell contains no internal organelles, a circumstance which simplifies isolation of the plasma membrane. Structural and functional changes that occur in the lens during the aging process and cataractogenesis suggest that an alteration in the lens plasma membrane is the key factor leading to the cataractous state (3,6,7,13,16,32,33,41). Over the past decade the majority of studies have focused on the cytoplasmic crystallins, lens membrane proteins, and lipids; but recently more interest has been given to lipid/protein interactions (37,39).

The objective of Chapter 1 is to examine lipid behavior in the human lens membrane, particularly the lipid order. Although differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), X-ray diffraction, and fluorescence spectroscopy have been used to study physical properties of plasma membranes (35,45), the method chosen for this study is fluorescence depolarization of *cis* and *trans* parinaric acid isomers (19,35,45). We hypothesize that the cataractous lens plasma membrane is less ordered (more fluid) than the age-matched normal plasma membrane. The "leaky" cataractous plasma membrane might allow for increased movement of metabolites, ions, waste products, and peroxides across the membrane which results in decreased transparency.

Prior studies and this study indicate that the lipid and protein composition of the cataractous and normal age-matched lens is basically the same (32); therefore, we propose that lipid/protein interaction contributes to the ordering of the lens plasma membrane. In order to examine such an interaction, it is necessary to reconstitute the lens membrane proteins with different lipid species. Chapter 2 specifically studies the effects of lipid structural order using fluorescence spectroscopic techniques.

Mammalian Plasma Membrane Composition

Since the plasma membrane of most mammalian cells is composed mainly of lipids and proteins, the relative proportions of these components vary in different cell types. Due to the amphipathic properties of biological lipids, they form a bilayer with their hydrophobic tails (fatty acids) residing in the interior portion of the membrane and their hydrophilic heads facing the inner and outer surface of the membrane. The lipid bilayer forms an impermeable barrier around the cell and limits the diffusion of substances into and out of the cytoplasm.

Membrane proteins associate with the lipid bilayer in two ways: (1) they either interact with the surface of the bilayer and are called peripheral or extrinsic proteins, or (2) they penetrate the bilayer and are called integral or intrinsic proteins. The amino acid composition of the protein helps to determine its interaction with the lipid bilayer. A more hydrophobic protein will tend to penetrate the bilayer whereas a more hydrophilic protein will interact at the surface of the bilayer. The interaction of hydrophobic and hydrophilic proteins with the lipid bilayer contributes to the asymmetry of the plasma membrane and are important determinants of various cell functions such as transport of molecules into and out of the cell, receptor binding, and cell recognition.

Properties of the Aging and Cataractous Human Lens Plasma Membrane

We are interested in the structure and interactions of the membrane lipids, particularly as they relate to cataractogenesis. The lipids of the normal and cataractous human lens plasma membrane consist mainly of phospholipids and cholesterol. Broekhuyse suggests that almost all lens lipid is located in the plasma membrane (6), whereas Andrews proposes that the cytoplasmic matrix associated with the plasma membrane may have a significant lipid content in the human lens fiber cell (3). Another study indicates that in the normal lens the majority of lipid resides in the plasma membrane, but in the age-matched cataractous lens a significant amount of lipid is found in a non-membranous fraction (32).

The major phospholipid component of the human lens is sphingomyelin, a very stable phospholipid which comprises approximately 45-65% of the total phospholipid content (6,12). Other phospholipids present are phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI). During the aging process, there is a continuous increase of sphingomyelin and a decrease in concentration of the other phospholipids. Tao and Cotlier report an increase in ceramide content for cataractous lenses of all ages as compared to normal age-matched lenses. Ceramides are intermediates in both the biosynthesis and catabolism of glycosphingolipids and sphingomyelin (41). There appears to be no significant difference in phospholipid composition for age-matched normal and cataractous lenses (6,32).

Cholesterol is the only sterol found in the human lens and comprises about half (by weight) of the lipid in the plasma membrane (8). Like sphingomyelin, cholesterol concentration increases in the age-matched normal and cataractous lens. The cholesterol concentration doubles between

25 and 75 years of age (an increase of 10.2 μg/yr) (15). The cholesterol to phospholipid molar ratio in the senile normal and cataractous lens nucleus is about 2:1. This is an unusually high ratio when compared to most other membranes. As with the phospholipids, there is no significant difference in the neutral lipid composition between normal and cataractous lenses (13).

The human lens plasma membrane also contains extrinsic and intrinsic proteins. The extrinsic proteins comprise about 4% of the total membrane proteins and contain polypeptides of 32 K and 35 K according to SDS-polyacrylamide gel electrophoresis, which have been characterized by isoelectric focusing (12).

The intrinsic proteins comprise at least 30% of the total membrane proteins. In the mature lens plasma membrane, there are two major intrinsic proteins (MIP). A polypeptide of approximately 26-27,000 daltons whose function has yet to be confirmed, but several studies propose that it is the major component of gap junctions in the human lens (11,2,17). Because the lens fiber cell contains no internal organelles to carry out metabolic processes, it is thought that the channels formed by gap junctions allow for the exchange of small molecules (less than 12,000 daltons) and metabolites between adjacent cells (18,28).

The other major intrinsic protein has a molecular weight of 22-23,000 daltons. MIP22 increases as the lens ages and is thought to be a degradation production of the 27,000 dalton species (11,33,34,46).

Cataractogenesis Studies

The integrity of the human lens cell plasma membrane must be maintained in order for the lens to function optimally. Loss of membrane integrity is thought to be a principal factor leading to cataractogenesis. The following studies explore possible alterations on the plasma membrane, which could lead to cataractogenesis.

Animal model studies show that cataract can be induced in rats by feeding them cataractogenic agents such as triparanol, galactose, or diazacholesterol. Triparanol feeding causes a reduction in the lens phospholipid content. Phosphatidylcholine and phosphatidylserine and cholesterol decrease, while sphingomyelin increases. There is also a loss of $(Na^+ + K^+)$-ATPase activity in the cortical and nuclear fractions of the lens. With galactose feeding, $(Na^+ + K^+)$-ATPase activity decreases, but total phospholipid and phosphatidylcholine increase rather than decrease and sphingomyelin and cholesterol remain constant. Diazacholesterol is extremely toxic and animals usually die before reaching the cataractous state. However, pre-cataractous animals show depressed phosphatidylcholine and cholesterol levels while sphingomyelin and desmosterol are elevated. These results show a positive correlation between changes in lens $(Na^+ + K^+)$-ATPase activity and phosphatidylserine content, but no correlation between the enzyme activity and other lens lipid levels can be made (27). Another study on Nakano mice suggest that there is a loss in $(Na^+ + K^+)$-ATPase activity resulting in a net uptake of $Na^+$, causing decreased transparency (4,23). Gupta also found a decrease in $(Na^+ + K^+)$-ATPase as well as $Mg^+$-ATPase activity in the human senile cataractous lens (20). Whether this decrease in enzymatic activity is a cause or the result of cataractogenesis is unknown.

It has been previously demonstrated that there is an alteration in the lipid composition of the aging and cataractous lens, but there appears to be no significant change that could lead to the cataractous condition. Several investigations indicate there is an increase in lysophospholipid concentration and that this results in lysis of the lens membrane (3,6,38). The use of $Ca^{++}$-containing buffers ($Ca^{++}$ is known to activate phospholipases) for membrane preparation or the use of old preparations, which have been frozen and thawed, could account for the artifactual appearance of lysophospholipids.

The majority of investigations have focused on the protein composition of the senile human lens, especially the water soluble crystallins in the cytoplasm and their aggregation with plasma membrane proteins. Spector (37) and Broekhuyse (10) suggest that in the senile human lens an insoluble extrinsic membrane protein aggregates with water soluble crystallins in the cytoplasm through disulfide linkages. These aggregates cause the refractory properties of the membrane to be altered leading to the decreased transparency.

Takemoto found a decrease in cysteine residues and an increase in half-cysteine residues as the cataractous condition becomes more severe which suggests that auto-oxidation of the -SH groups of cysteine to S-S cysteine. This alteration in amino acid composition may contribute to cataractogenesis (39).

Recently more interest has been focused on the 27,000 dalton polypeptide (MIP), the putative gap junction protein. Studies conducted on 90 day Nakano mice found no 27,000 dalton protein in plasma membranes of cortical fiber cells which correlates with a decrease in gap junctions may be linked to cataract development in these mice (40). In the aging human

lens plasma membranes there is a decrease in the 27,000 dalton polypeptide and an increase in the 23,000 dalton polypeptide (33), but no correlation has been made to a decrease in gap junctions.

The 27,000 dalton polypeptide is a very hydrophobic species, but little is known about the lipid composition of the human lens gap junction. Alcala has studied the lipid composition of chick lens fiber cell gap junctions, and he found an unusually high cholesterol/phospholipid molar ratio of 3:1. He also found that the gap junctions contain about 57% of the total fiber cholesterol and 53% of the total fiber sphingomyelin (2). Cholesterol and sphingomyelin tend to have an ordering effect on lens plasma membranes and since human lens plasma membranes have an unusually high content of these two lipids, it is not surprising that they are highly ordered and have even been described as entirely rigid membranes (44). Proteins are known to have an ordering effect on plasma membranes (21,24); therefore, the unique lipid/protein interactions involved in the human lens cell gap junction may be a key factor in maintaining the integrity of lens fiber cell. An alteration in this lipid/protein interaction of the gap junction may be a step in the event(s) leading to cataractogenesis. The following chapters represent the preliminary approach to test this hypothesis.

Reconstitution

Reconstitution is an ideal method for studying lipid/protein interactions. By isolating the protein under investigation and reconstituting it with lipids of known composition, researchers can simplify a complex biological system and study it in detail. Reconstitution is achieved by either removal of detergent from detergent-lipid-protein complexes or by perturbing liposomes in order for proteins to be inserted.

There are five methods available for reconstitution--black lipid film, detergent-catalyzed incorporation, sonication, detergent dialysis, and detergent removal (22). The method of choice depends on the membrane protein to be studied because each protein reacts differently to the various reconstitution methods available. For example, $(Na^{+} + K^{+})$-ATPase from electric eel is sensitive to detergents, and sonication is the only method that yields active vesicles (30). Conversely, the $Ca^{++}$ pump of the sarcoplasmic reticulum has been actively reconstituted with cholate dialysis, but optimal transport occurs when a mixture of deoxycholate and cholate is used (25,29).

There are other factors to consider when developing a workable reconstitution system. The procedure for isolation and purification of the membrane protein must not alter or denature the protein. The lipid requirement for successful reconstitution depends on the reconstitution method selected and the requirements of the protein function (14). The orientation of the protein in the reconstituted vesicle must be considered too. Once the system is perfected, valuable information can be obtained about membrane lipid/protein interactions and functions.

To date, no one has successfully reconstituted the human lens gap junction protein, although Girsch and Peracchia have incorporated MIP 26 isolated from calf lens into liposomes in order to study permeability regulation by calmodulin. These investigators used a sonification/ resuspension method for reconstitution of the MIP 26 and formed gap junctions. They found they could add calmodulin to the reconstitution mixture and produce channels which could be opened and closed depending on the presence or absence of $Ca^{++}$ (17). The results presented in Chapter 2 concerning reconstitution of the human lens membrane major intrinsic protein will further the development of a functional gap junction model system.

# SECTION III

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# SECTION IV

## CHAPTER 1: EVIDENCE FOR REDUCED LIPID ORDER IN PLASMA MEMBRANES FROM CATARACTOUS HUMAN LENSES

ABSTRACT

Membrane preparations from normal and cataractous human lenses were prepared by a procedure which minimally disrupts membrane lipid structure. Fluorescence depolarization of *cis* and *trans*-parinaric acid probes was measured as a function of temperature in membranes, lipid extracts, and phospholipids. These measurements indicated that membranes from cataractous lenses were less ordered (more fluid) than equivalent membranes from normal, age-matched control lenses. No significant differences could be detected between normal and cataractous membrane total lipid extracts, or between normal and cataractous membrane phospholipids. These observations imply that membrane lipid-protein interactions play a significant role in determination of lens membrane structure, and that these interactions are altered in cataractous lens membranes.

## INTRODUCTION

Disruption of lens fiber cell membrane structure and/or function is suggested to be an early event in the development of human senile cataract. Evidence supporting this hypothesis includes:

1. A small but significant increase in the oxidation of membrane proteins in cataractous lenses (6,9).
2. Altered distribution of cholesterol and phospholipid into "non-membranous" fractions in cataractous lenses (16).
3. Loss of $Na^+$, $K^+$ -ATPase activity in developing rat lens cataracts (15).
4. An increase in ceramide content of cataractous human lens membranes (21).

The exact relationship of these changes to cataractogenesis is uncertain, but it is becoming clear that changes in membrane structure may play an important role in the process. We examined the motion and distribution of lens membrane lipids, using recently developed spectroscopic techniques, in order to determine if these parameters are altered in cataractous lenses. However, many commonly used methods for preparation of lens membranes rely on chaotropic agents such as urea or guanidine (1,9,14,16,18,22) to remove the so-called extrinsic or matrix proteins. The concentrations of these agents in most membrane isolation procedures is very likely to disrupt the original membrane lipid structure (17) as evidenced by the report that 25-40% of the phospholipid is solubilized from human lens membranes after urea treatment (6). Additionally, the major intrinsic protein (MIP) of the lens membrane cannot be extracted into organic solvents unless the membranes are first treated with a chaotropic agent (6). This has been interpreted as evidence for MIP-matrix interactions, but is equally likely

to be evidence for disruption of lipid-protein interactions after urea treatment.

Considering the fact that most previous investigations have concentrated on lens protein content, it is not surprising that such membrane isolation protocols are not optimized for preparation of membranes whose lipid structure and content closely resembles that of the intact cellular membrane. However, at least one previously published protocol, based on sucrose gradient centrifugation (4), is likely to yield membrane fractions that are minimally altered in lipid structure. We have adapted this procedure to isolate pure lens membranes from normal and cataractous lenses, and have used these preparations in fluorescence depolarization experiments in order to examine the motion and distribution of lens membrane lipids.

## MATERIALS AND METHODS

### Membrane preparation

Normal lenses were obtained from eye banks, and cataractous lenses were obtained from local ophthalmologists; cataractous lenses all showed significant opacification in nuclear and/or cortical regions. Both types of lenses were frozen immediately after removal, and thawed immediately before homogenization. Decapsulated lenses were placed in a glass tube and homogenized in buffer (1.0 mM Tris/HCl, pH=8.0, 1.0 ml per lens) for two minutes using a tight fitting Teflon pestle at 0-4°C. Homogenates were then stirred on ice for 20 minutes, rehomogenized as above, and resuspended in 40 ml Tris buffer. After centrifugation at 3015 g in an SS34 Sorvall rotor for 10 minutes, the supernatant was carefully decanted. This post-nuclear supernatant was centrifuged 1h at 39,049 g in a JA-20 rotor and Beckman J-21 centrifuge. The pellet was resuspended in 40 ml Tris buffer, and the washing procedure was repeated a minimum of four times, or until the $A_{280}$ of the supernatant was less than 0.05. The final pellet was resuspended in 1.5 ml of 60% (w/w) sucrose in Tris buffer, placed in a Beckman SW50.1 tube, and sequentially overlaid with 0.8 ml of the following sucrose/Tris solutions: 40%, 35%, 29% and 20%. Tubes were filled to the top with Tris buffer, and centrifuged for 12-15 hours at 178,720 g in the SW50.1 rotor. Membrane bands at the 35-40% sucrose interface were collected, resuspended in buffer, and centrifuged for 1h at 141,188 g in a Beckman 50Ti rotor. These purified membrane bands were resuspended in a minimal volume of Tris buffer and used for further analysis.

### Lipid analysis

Membrane fractions and lens homogenates were resuspended in sufficient chloroform/methanol (2/1) to give a single phase and was vigorously stirred

on a Vortex mixer for 2 minutes. Particulate matter was filtered out with a glass wool-plugged Pasteur pipet; the glass wool was subsequently re-extracted with 5.0 ml of chloroform/methanol (2/1) mixture. This extract was also filtered, and the glass wool was rinsed first with 5.0 ml methanol and then with 10.0 ml chloroform. Extracts were pooled, and 0.88% KCl in glass distilled water was added to give a final ratio of 2/1/1 chloroform/methanol/water. After centrifugation at 1000 g for 5 minutes to separate the phases, the lower phase was removed and evaporated under $N_2$, was resuspended in 1.0 ml chloroform and was assayed for lipid phosphorus and cholesterol. This procedure routinely extracted greater than 95% of the lipid phosphorus and cholesterol present in the original sample, and no lipid phosphate could be detected in the upper phase after centrifugation. Lipid phosphate was measured by the method of Ames (2), and cholesterol was measured by the method of Gamble, et al. (8). Phospholipids and neutral lipids were separated by silicic acid column chromatography, and two dimensional thin layer chromatography of phospholipids was performed according to the procedure of Freter, et al. (7).

Fluorescence depolarization

Fluorescence depolarization of cis and trans parinaric acid isomers (cPnA and tPnA, kindly provided by Dr. R.D. Simoni, Department of Biological Sciences, Stanford University) was measured on a Spex Fluorolog spectro-fluorometer. Sample heating and cooling was regulated by a circulating water bath and a temperature programmer. Sample temperature was monitored by means of a thermocouple inserted directly into the sample cuvette. The polarization ratio ($I_{11}/I_1$) was calculated and smooth curves were derived by computer analysis as previously described (23). When necessary, corrections for scattering depolarization were calculated by the

method of Lentz, et al. (13). Artificial liposomes were prepared from lipid extracts using an ethanol injection method (24). All liposome preparations were prepared immediately before analysis, and the buffer used in all fluorescence experiments was 10 mM HEPES, 50 mM KCl, pH=7.2. The limiting value of the polarization ratio ($I_{11}/I_1$) was 2.6 ± 0.05, for fluorescein in alkaline glycerol at 10°C(19).

Other methods

Protein was assayed as previously described (23), and bovine serum albumin was used as a standard. SDS-polyacrylamide gel electrophoresis was performed by J. Hansen of this department, using the procedure of Laemmli (12). Urea extracted lens membranes were prepared as previously described (1). All buffers were made up using glass-distilled water, and, unless otherwise indicated, all reagents were from Sigma.

## RESULTS

### Membrane Isolation

Normal and cataractous lens membrane preparations obtained as described above were analyzed for sterol, phospholipid, and protein content. Such analyses revealed no significant differences in final yield between the two types of lenses. Approximately 65% of the phospholipid and 30% of the sterol could be recovered in the 35-40% sucrose interface in both cases, using either single lenses or pools of age-matched lenses. The sterol/phospholipid ratio ranged from 1.0 to 2.46 (mol/mol) for normal membranes, and from 1.5-2.77 for cataractous membranes; no significant differences could be detected between age-matched normal and cataractous lenses. The phospholipid/protein ratio (μmol phosphate/mg protein) was 0.38-0.46; no consistent differences could be detected between normal and cataractous lenses. These data are in agreement with many previous reports (3,5,6,15,16).

SDS polyacrylamide gel electrophoresis patterns of single lens membrane preparations, and urea-treated membrane preparations, are shown in Fig. 1. The major protein bands are at 27K and 23K, corresponding to the major intrinsic protein. Contamination of the membrane preparations with lower molecular weight peptides was approximately equal in membranes derived from sucrose gradient centrifugation and the urea extraction. We conclude that sucrose gradient preparation of lens membranes under these conditions yields membranes of equal or greater purity compared to the standard urea extraction methods.

### Fluorescence polarization

Lens membranes were prepared as described above, and aliquots containing approximately 50 nmol of lipid phosphate were used for

fluorenscence polarization analysis (11,20,23). 0.5 nmol of cis or trans PnA and 0.1 nmol of BHT were added from concentrated ethanolic stocks. Fluorescence intensity parallel and perpendicular to the vertically polarized excitation was monitored as these preparations were heated from 8°C to 45°C at a rate of approximately 0.75 C°/min; equivalent results were obtained when samples were cooled over this temperature range. We often observed that lens membranes samples heated above 50°C did not exhibit consistent fluorescence depolarization profiles; the absolute value of the polarization ratio was always lower when compared to non-heated membranes. This phenomenon was regularly observed using cataractous lens membranes but occasionally could be observed using normal lens membranes as well. Such irreversible thermal effects were not observed with lipid extracts (see below). We interpret these observations as indicative of irreversible thermal alteration of membrane proteins or membrane protein/lipid interactions above 50°C. Lipid analysis revealed no differences in phospholipid head group composition after membrane heating. It is unlikely that generation of lysophospholipid can explain these results; however, the exact nature of this thermal effect remains to be determined. All experiments were therefore performed on membranes which had not been heated above 45°C.

Fluorescence depolarization of cPnA and tPnA is a representative experiment using normal and cataractous membranes is shown in Figure 2. The value of the polarization ratio at 37°C for other membrane preparations is also given in TABLE 1. The polarization ratio, or fluorescence intensity emitted parallel to the vertically polarized excitation light divided by the fluorescence emitted perpendicularly ($I_{11}/I_1$), has been shown to be related to the degree of lipid structural order in a number of

model and biological membranes (11,20,23). The absolute value of the polarization ratio is directly related to the degree of lipid order; a high value (2.2-2.5) is characteristic of solid or gel-phase lipids (19,20). Additionally, the partitioning behavior of the two isomers of PnA affects the polarization ratio: tPnA preferentially partitions in solid lipid in mixed bilayers, while cPnA is more evenly distributed between solid and fluid (liquid-crystalline) phases in mixed bilayers (20). For this reason, tPnA is more useful for detecting small (5%-40%) percentages of solid phase lipid, while cPnA is more useful in determination of the average membrane structure. Finally, both probes can be used to detect lipid phase transitions, or changes in the structural order of membrane lipids. A gel to liquid-crystalline phase transition is characterized by a sharp decrease in the polarization ratio over a relatively small temperature range in model membranes (20). Equivalent changes in polarization ratio have been shown to be due to phase separations or phase transitions in biological membranes (23). We can analyze two separate parameters in interpretation of PnA fluorescence polarization data; both the absolute value of the polarization ratio and the rapid changes in the ratio are important indices of membrane lipid structure. As can be seen from Fig. 2, the probes detect significantly more solid lipid in the normal membrane than in the cataractous membrane. Alternatively, it could be stated that the cataractous membranes are more "fluid", if we define increased "fluidity" as corresponding to a higher rate of rotational motion of the probe molecule. Fluorescence polarization spectroscopy of parinaric acid is sensitive to this rotational motion; other types of molecular motion, such as lateral diffusion (11), cannot be measured by this technique. Thermal profiles of PnA fluorescence polarization in liposomes prepared from lipid

extracts are shown in Fig. 3; equivalent measurements on lens phospholipid preparations are shown in Fig. 4. We could detect no consistent differences between the normal and cataractous total lipid preparations, with either cPnA or tPnA (Fig. 3). The small differences shown in this figure were not reproducible from preparation to preparation. Likewise, examination of a number of phospholipid liposome preparations, using both probe species, revealed no consistent differences between normal and age-matched cataractous lens lipids, with one exception. At higher temperatures (45-55°C), phospholipid preparations from normal lenses had consistently higher polarization ratios, using either probe, than equivalent preparations from age-matched cataract membranes.

The polarization values obtained at low temperature using parinaric acid probes in these preparations were very high, and showed no reproducible differences between normal and cataractous membrane lipids. It should be noted that the polarization ratio, at all temperatures and with both probes, was always lower in total membrane lipid extracts than in the original lens membrane preparation. Finally, the sharp increase in the polarization ratio at 45-50°C in both types of phospholipid preparations was detected with both isomers, indicating that a major lipid phase transition was occurring at this temperature. This phase transition was not detected in the total lipid extracts (Fig. 3); we propose that this is due to the high sterol content of the total lipid preparations. Sterol was the major component removed by the silicic acid column although traces of triglycerides and sterol esters were also found. Sterol is known to suppress phase transitions in many model and biological membranes (7,19,23).

Lipid Analysis

Sterol and phospholipid analyses of normal and cataractous membranes are shown in Table I. The major phospholipid in these membranes was sphingomyelin, which accounted for 45-65% of the total lipid phosphate. This is in agreement with many previous reports (5,6,15,16). No significant amount of lysophospholipid could be found in fresh preparations, even in very old lenses. Lysophospholipid, mostly lysophosphatidylethanolamine and lysophosphatidylserine, was often found in membrane preparations which had been frozen and thawed (data not shown). It is possible that previous reports (3,5,6,15,16) of high lysolipid concentrations in human lens membranes are due to the use of frozen preparations. An alternative explanation might be the use of $Ca^{++}$ -containing buffers for membrane preparation. The homogenization, centrifugation, and analysis of the membrane preparations used in the present study were all performed in $Ca^{++}$ -free buffer, in order to inhibit endogenous $Ca^{++}$ -dependent phospholipases. It can be seen from Table 1 that there were no significant differences between cataractous membrane lipids and those of age-matched controls, again in agreement with previous reports (3,5,6,15,16).

## DISCUSSION

The fluorescence polarization data presented in Fig. 2 and Table I indicate a significant difference in membrane lipid physical properties between normal and age-matched catatactous lens membranes. Since the parinaric acid probes are hydrophobic, we interpret these data as evidence for increased membrane lipid "fluidity" in the cataractous lens membrane, regardless of age. This increased fluidity cannot be attributed to the lipids alone, as lipid extracts and phospholipids from normal and cataractous membranes present very similar thermal profiles of PnA fluorescence depolarization. (Figs. 3 and 4). Additional evidence for this interpretation comes from the similar lipid composition of the normal and age-matched cataractous lens membranes (Table I). We therefore propose that the increased lipid fluidity detected in the cataractous lens membrane is due to altered lipid/protein interactions, and that these interactions serve to increase rigidity in the lens membrane in the normal fiber cell. It should be pointed out that the polarization ratio of tPnA at 37°C in normal lens membranes is very high, near the experimental maximum of 2.6. Such a high polarization ratio at the physiological temperature has not been previously observed in biological membranes. Equivalent measurements previously reported include a value of 1.65 for mouse LM cell plasma membranes (23), 1.7 for human erythrocyte ghosts (24), and 1.75 for bovine corpus luteum membranes (11). Thus, the ratio of 2.3-2.4 observed in the normal membranes indicates a very high degree of structural order in these preparations (22). The cPnA polarization ratio is also very high for a biological membrane. The lipid composition of these membranes is consistent with a high degree of structural order. Previous workers have often noted a positive correlation between high sphingomyelin and sterol

content and low lipid fluidity (22). The lower polarization ratio observed with both isomers in cataractous membranes can thus be interpreted as evidence for a decrease in this high degree of structural order, or an increase in fluidity.

The polarization ratio of liposomes prepared from total lipid extracts of these membranes was consistently lower than that of the original membranes and also very similar for normal and age-matched cataractous lens lipids. This decrease in polarization ratio, indicative of a decrease in membrane lipid order, can be interpreted as evidence for the presence of "rigidity inducing" lipid/protein interactions in the original membrane. Alternatively the original membrane contained domains of high structural order, perhaps due to bilayer asymmetry. These domains could be disrupted by the extraction procedure, resulting in an apparent decrease in order in the liposomes prepared from such extracts. Presently we cannot prove or disprove either hypothesis; however, an observation in favor of the first hypothesis is the fact that fluorescence polarization ratios of the parinaric acid probes in these membranes decreased markedly in samples heated above 50°C. Thermal denaturation of membrane proteins could result in altered lipid/protein interaction and could be the cause of altered probe motion and/or distribution, resulting in an altered polarization ratio. Further experiments are in progress to more critically address this question.

The data presented in this report are consistent with the hypothesis that altered lipid-protein interactions and membrane disruption are hallmarks of the cataractous state (9,16). Freeze-fracture electron microscopy of preparations similar to those used in this study has indicated that regional specializations, such as intercellular junctional

complexes, are retained in the purified membrane bands obtained from sucrose gradients (4). These regional specializations may be responsible for the high degree of lipid/protein interaction indicated by our data. Since the major protein in these preparations is the 23K and 27K MIP, the putative gap junctional protein, it is a likely candidate for this sort of lipid/protein complex.

The contribution of lipid structural disruption to cataractogenesis remains to be fully elucidated. It seems possible that improper alignment of gap junctional complexes could result in changes of membrane permeability. Such permeability changes have been previously implicated as potential initiators of cataractogenesis (10). Specific lipid/protein interactions may be required to maintain membrane and junctional integrity in this highly specialized organelle. Alteration of these specific interactions may be an important step in the initiation of membrane dysfunction and subsequent cataractogenesis.

Figure 1: Human lens membranes were prepared from individual lenses as described in the text. Urea-extracted membranes were prepared as described in Ref. 1. Samples were analyzed for protein, and approximately 50 µg of protein was applied to lanes 1-6 (sucrose gradient preparations); approximately 250 µg of protein from the urea-extracted membranes was applied to lanes 7 and 8. Samples were electrophoresed on 10% acrylamide gels, stained with Coomassie blue, and destained as decribed (12).

Lane 1, urea extracted cataractous human lens membranes

Lane 2, urea-extracted normal human lens membranes

Lanes 3-5, sucrose gradient-derived normal human lens membranes

Lanes 6-8, sucrose gradient-derived cataractous human lens membranes

MP26 = 26K membrane protein

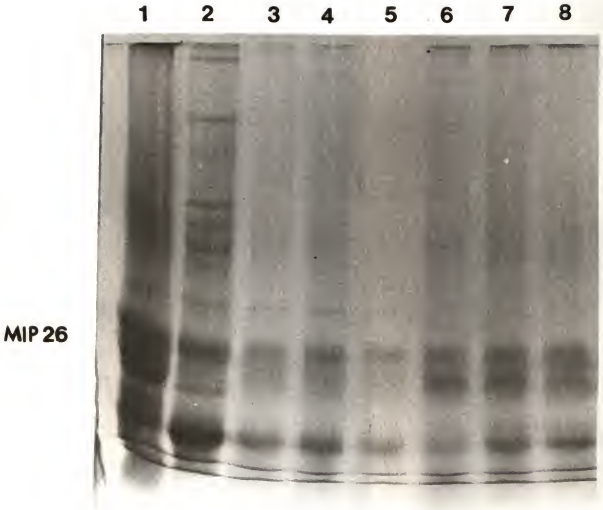

1
2
3
4
5
6
7
8
MIP 26

Table 1: Membranes prepared by sucrose gradient centrifugation as described in Materials and Methods were extracted with chloroform/methanol (2/1). Aliquots were assayed for sterol and for phosphate, and an aliquot totaling 100 nmol of lipid phosphate was subjected to two-dimensional thin-layer chromatography as described in ref. 7. After development, the thin layer plate was sprayed lightly with 50% $H_2SO_4$, and charred overnight at 150°C. Spots corresponding to genuine phospholipid standards were scraped from the plate and assayed for phosphate as described. Recovery from the plates was approximately 80-90%.

PC=phosphatidylcholine, PE=phosphatidylethanolamine,

PI=phosphatidylinositol, PS=phosphatidylserine

PL=phospholipid, SM=sphingomyelin, N.D.=not determined

$I_{11}/I_1$ at 37°C was determined as described in Materials & Methods

TABLE 1

| Normal lens Membranes (# lenses pooled) | Sterol Phospholipid (mol/mol) | % SM | %PC | %PE | %PS | %PI | %Other PL | $I_{11}/I_1$, using tPnA, at 37°C |
|---|---|---|---|---|---|---|---|---|
| Normal, pooled | | | | | | | | |
| 43-54 (3) | 2.46 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 2.31 |
| 50-56 (2) | 2.2 | 52 | 2.5 | 10.7 | 15 | 1.0 | 19.0 | 2.35 |
| 50-58 (2) | 1.87 | 56 | 2 | 12 | 6 | 2 | 22 | N.D. |
| 54-61 (4) | 1.58 | 53 | 2.6 | 8.5 | 13 | 4 | 19 | N.D. |
| 54-61 (4) | 1.39 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 2.16 |
| 60-61 (2) | N.D. | 47 | 4 | 11 | 10 | 2 | 26 | N.D. |
| 61 (2) | 2.0 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 2.28 |
| INDIVIDUAL LENSES | | | | | | | | |
| 38 | 2.2 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 36 | 1.7 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 18 | 1.0 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Cataract Lens Membranes (# lenses pooled) | | | | | | | | |
| 45-52 (3) | 1.96 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 2.13 |
| 48-52 (2) | 1.5 | 56 | 3 | 13 | 6 | 1 | 21 | N.D. |
| 54-64 (3) | 1.64 | 60 | 2 | 7 | 12 | 1 | 18 | 1.96 |
| 55-56 (2) | N.D. | 42 | 3 | 12 | 14 | 5 | 24 | 1.93 |
| 65-66 (2) | 2.27 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 1.93 |
| 81-89 (6) | 2.77 | 60 | 4 | 5 | 9 | 2 | 20 | 2.07 |
| INDIVIDUAL LENSES | | | | | | | | |
| 55 | 1.8 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 61 | 2.4 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 62 | 1.9 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

Figure 2: Fluorescence polarization ratios of parinaric acid probes in normal and cataractous human membranes. Membranes were prepared by sucrose gradient centrifugation as described in the text. Fluorescence depolarization of parinaric acid isomers was measured as described, and smoothed curves were calculated by computer analysis as described in ref. 23. Normal lenses used for this experiment were ages 50-56, cataractous lenses were ages 54-64. Three lenses were pooled to obtain the membranes used in the experiment shown. Data were obtained by heating the sample from 8°-45°C, at a rate of 0.75 C° per minute, while monitoring fluorescence emission parallel and perpendicular to the vertical excitation beam.

O = normal membranes, cis-parinaric acid probe

● = cataractous membranes, cis-parinaric acid probe

Δ = normal membranes, trans-parinaric acid probe

▲ = cataractous membranes, trans-parinaric acid probe

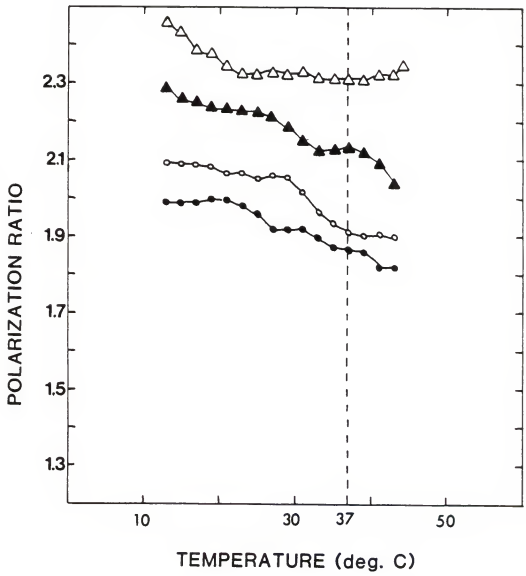

POLARIZATION RATIO
2.3
2.1
1.9
1.7
1.5
1.3
10
30
37
50
TEMPERATURE (deg. C)

Figure 3: Fluorescence polarization ratios of parinaric acid probes in normal and cataractous human lens membrane total lipid extracts. Membranes were prepared and extracted as described in the text; 50 nmol of phospholipid was used to prepare liposomes in buffer as described in ref. 24. Conditions and lens ages were as described in legend to Figure 2.

O = normal membranes, cis-parinaric probe

● = cataractous membranes, cis-parinaric probe

Δ = normal membranes, trans-parinaric acid probe

▲ = cataractous membranes, trans-parinaric acid probe

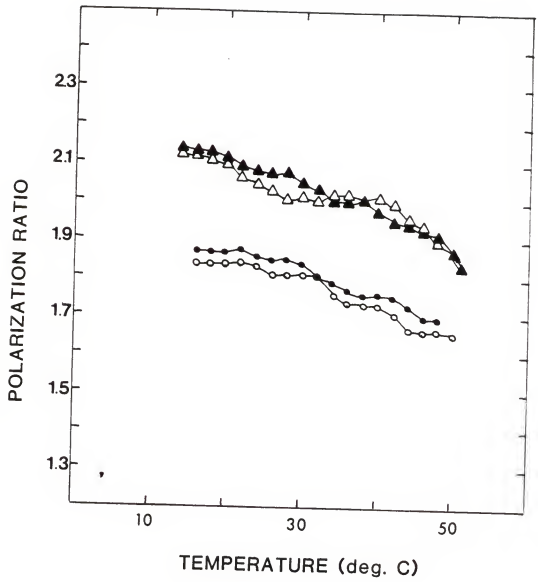

POLARIZATION RATIO
2.3
2.1
1.9
1.7
1.5
1.3
10
30
50
TEMPERATURE (deg. C)

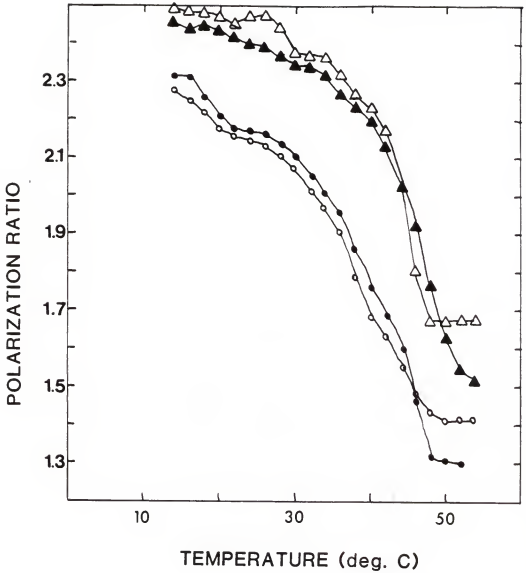
Figure 4: Fluorescence polarization ratios of parinaric acid probes in normal cataractous human lens phospholipids. Membranes were prepared and extracted as described in the text, phospholipids were prepared by silicic acid column chromatography. 50 nmol of phospholipid were used to prepare liposomes as described in ref. 24. Conditions and lens ages were described in the legend to Figure 2.

○ = normal membrane phospholipids, cis-parinaric acid probe

● = cataractous membrane phospholipids, cis-parinaric acid probe

△ = normal membrane phospholipids, trans-parinaric acid probe

▲ = cataractous membrane phospholipids, trans-parinaric acid probe


POLARIZATION RATIO
2.3
2.1
1.9
1.7
1.5
1.3
10
30
50
TEMPERATURE (deg. C)

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SECTION V

# CHAPTER 2: MEMBRANE LIPID ORDER IN RECONSTITUTED HUMAN LENS MEMBRANES

ABSTRACT

Isolation and reconstitution of the 27 K and 23 K major intrinsic protein with the total lipid extract of the original lens membrane and single species phospholipids was described. Previous solubilizing methods used chaotropic agents such as urea, guanidine, citraconic anhydride, or chloroform-methanol (3,8). The ionic detergent sodium cholate was used for both lens protein solubilization and reconstitution of the lens membrane protein. Results indicated that this method recovered 95% of the protein without any significant alteration of the protein species. The reconstitution method of the major intrinsic protein with different lipids indicated there was a stable association of the protein with the lipid, and the degree of lipid ordering depended on the lipid species used for reconstitution.

## INTRODUCTION

Results from Chapter 1 indicate that the cataractous plasma membrane was less ordered (more fluid) than the normal lens plasma membrane. Because the lipid and protein composition of age-matched normal and cataractous lenses were similar, this suggested an alteration in the lipid/protein interaction of the cataractous membrane, rendering it more fluid.

Several methods have been developed for reconstitution of membrane proteins (5,7). The method of choice depends on the unique properties of the biological membrane under investigation. The purpose of this study is to describe a method of reconstitution of the major intrinsic protein (MIP), 23 K and 27 K, isolated from the human lens plasma membrane. This study described a new method for solubilizing the major intrinsic protein with cholate and then reconstituting the protein with a cholate-dialysis method as described by Racker (7).

We reconstituted the major intrinsic protein with egg phosphatidylcholine (Egg PC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and membrane lipid extract to determine if there were any differences in the degree of lipid/protein association. These preparations were also used in fluorescence depolarization experiments to examine the effect of the major intrinsic protein on the motion and distribution of the different lipid species (2,9,10,11,12).

## MATERIALS AND METHODS

### Membrane Preparation

Normal and cataractous lenses were used to prepare membranes by the method previously described in Chapter 1.

### Lipid Extraction

Membranes were extracted and lipids stored as previously described in Chapter 1. Egg PC was purchased from Sigma and DPPC was synthesized by Dr. Ruth Welti, Biochemistry, University of Kansas Medical Center.

### Protein Isolation

Normal and cataractous membranes were dissolved in 1mM TRIS/HCl (pH=8) containing 1% sodium cholate (w/v). These solutions were dialyzed at 4°C for 2 days with four changes of TRIS/Cholate buffer in order to remove phospholipids and cholesterol from the membranes. Dialyzer tubing (m.w. cutoff 12,000) was purchased from Arthur H. Thomas.

### Reconstitution

Sodium cholate solubilized proteins were added to sodium cholate (1%) solubilized lipids (phosphatidylcholine, dipalmitoylphosphatidylcholine, and total lipid extracts from original lens membranes) at a phospholipid (mM) /protein (mg) ratio calculated for the original lens membrane, usually 2.3. The preparation was dialyzed overnight against 1mM TRIS/HCl with no cholate. Larger unilamellar vesicles were obtained by freezing and thawing three or four times.

### Lipid and Protein Analysis

We previously used the Ames method (1) for lipid phosphate measurements. Since we found that cholate interfered with this method, we used the malachite green phosphate assay, adapted from Muszbek (6). Lipid composition was determined by two-dimensional thin layer chromatography.

Protein was assayed and SDS-polyacrylamide gel electrophoresis was performed as previously described in Chapter 1.

Fluorescence Depolarization

Fluorescence depolarization of cis and trans parinaric acid isomers was measured as described in Chapter 1. Polarization ratios were calculated for membranes isolated from chick and human lenses, liposomes containing only lipid, and reconstituted liposomes containing lipid and major intrinsic protein.

As stated in Chapter 1, cis-parinaric acid partitions between solid and fluid phases of the lipid bilayer and gives an overall picture of the membrane structure. Trans-parinaric acid partitions with the more solid lipids of the lipid bilayer. These two free fatty acid probes can be used to determine the temperature of gel to liquid-crystalline phase transitions, to examine the relative rotational mobility of these probes in the various membranes, and to detect the appearance of small domains of relatively immobile lipid.

## RESULTS AND DISCUSSION

### Protein Analysis

Extraction of chick lens membranes using 1% sodium cholate, indicated that 98% of the phospholipid and 97% of the sterol was removed. Protein recovery was approximately 95%. Similar results were obtained when human lens membranes were solubilized with 1% sodium cholate.

SDS-polyacrylamide gel electrophoresis patterns of the original human lens membrane preparation, the cholate solubilized protein, and the reconstituted protein are shown in figure 1. The major protein bands are at 27 K and 23 K, which corresponds to the major intrinsic protein. There was no significant alteration of the protein species; therefore, we concluded that the major intrinsic protein could be isolated by cholate solubilization and dialysis. This method was faster and less chaotropic than other methods such as urea or chloroform-methanol extraction of proteins. We suggest the cholate solubilized protein be reconstituted as soon as possible in order to avoid denaturation of the protein by prolonged contact with the detergent.

### Lipid Analysis

Two dimensional thin layer chromatography (TLC) of phospholipids was performed on the membrane total lipid extract, cholate-solubilized protein, and reconstituted protein. Lipid phosphate assay of TLC spots indicated that the major lipid in the membrane lipid extract was sphingomyelin. This in agreement with previous reports (4,11).

Phosphate assays of cholate-solubilized protein were negative; however, thin layer chromatography revealed that there was a small amount of sphingomyelin present. Perhaps the extremely hydrophobic nature of the

major intrinsic protein made it difficult to remove all the lipid from the protein.

A comparison, using TLC, of the lipid composition of the membrane lipid extract and liposomes containing membrane lipid extract and major intrinsic protein indicated that sphingomyelin was, as expected, the predominant lipid and that there was a loss of certain phospholipid species such as phosphatidylserine and phosphatidylcholine during reconstitution.

A possible explanation for the loss of certain lipid species is that such a small amount is present when reconstituting the major intrinsic protein which is not detectable on a TLC plate. Alternatively, the major intrinsic protein prefers certain lipids, such as sphingomyelin, to the exclusion of others when forming liposomes.

When liposomes were formed from lipid alone, using cholate dialysis, there was a significant loss of lipid, as much as 65%; however, when the lipid was used for reconstitution of the major intrinsic protein there was very little lipid lost, see Table 1. These results suggested that the lipid and protein do interact favorably to form liposomes. Studies are currently in progress to determine if there is a minimum concentration of lipid necessary to prevent the loss of lipid from the dialysis bag during liposome preparation. The lipid concentration will probably vary depending upon the lipid species being used for reconstitution.

Fluorescence Depolarization of Reconstituted Liposomes

One way to determine the success of a reconstitution method is to compare polarization ratios of liposomes of the original membrane to reconstituted liposomes. Figure 2A shows the similarity in polarization ratios of chick lens membrane liposomes and reconstituted liposomes containing chick lens lipid and chick lens protein. These results

indicated that solubilized chick lens protein and lipid can be isolated and then reconstituted with cholate, and can exhibit polarization ratios almost identical to the original chick lens membrane.

In order to study the ordering effect of the chick lens major intrinsic protein, it was reconstituted with egg phosphatidylcholine (egg PC), Figure 2B. The higher polarization ratio of the liposomes which contain protein showed that the addition of protein ordered the highly fluid egg PC.

Further studies on lipid ordering were performed on the human lens protein (MIP 27) by reconstituting with egg PC, DPPC, and human lens membrane lipid extract. Figure 3 compares polarization ratios (using cis-parinaric acid) for the original lens membrane liposomes, total lipid extract liposomes, and liposomes containing the total lipid extract and lens major intrinsic protein. Even though the major intrinsic protein was reconstituted with the total lipid extract at a ratio equivalent to the original membrane 2.3 (mg protein/μM phospholipid), the polarization ratio was higher than the original lens membrane. A possible explanation is that the cholate-solubilized protein had sufficient residual sphingomyelin to increase the ordering. As stated previously, sphingomyelin is known to have an ordering effect (11).

Figure 4 compares polarization ratios using trans-parinaric acid for liposomes containing MIP 27 with egg PC or with DPPC. It is apparent that the protein orders the egg PC and DPPC. It can be noted that the polarization ratios for DPPC are much higher than egg PC because DPPC is a pure phosphatidylcholine and contains identical saturated fatty acyl chains (16:0) which allows for greater packing of the chains resulting in a more ordered membrane (9).

MIP27 reconstituted with DPPC is shown in figure 5. Cis parinaric acid was used for fluorescence studies and the polarization profile showed a transition occurring at 41°C for pure DPPC liposomes. This is the reported transition temperature (Tc) for DPPC (9). When MIP27 was added to DPPC, the Tc increased to 46°C which indicated that the palmitic acid chains were more tightly packed (more ordered) and a higher temperature was needed to go from a gel to a liquid-crystalline phase. It was interesting that MIP27 increased the order of an already highly ordered bilayer.

In Chapter 1, we concluded that cataractous lens plasma membranes are more fluid than normal age-matched lens membranes. The reconstitution method developed in this study can be applied to future studies of lipid order in normal and cataractous human lenses in order to determine whether cataractous lens proteins behave differently from normal lens proteins in reconstituted liposomes. Through reconstitution we can manipulate the lipid and/or protein components and hopefully determine why the plasma membranes of cataractous lenses are more fluid.

Figure 1: Human lens membranes were prepared as described in Chapter 1. Cholate-solubilized protein and reconstituted protein were prepared as described in Materials and Methods. Samples were analyzed for protein and approximately 50 ug of protein was applied to each lane. Samples were electrophoresed on 10% acrylamide gels, stained with Coomassie blue, and destained as described in Ref. 12, Chapter 1.

Lane 1, Cataractous human lens membrane

Lane 2, Normal human lens membrane

Lane 3, Cholate-solubilized major intrinsic protein from cataractous human lens membrane

Lane 4, Cholate-solubilized major intrinsic protein from normal human lens membrane

Lane 5, Cataractous major intrinsic protein reconstituted with dipalmitoylphosphatidylcholine

Lane 6, Cataractous major intrinsic protein reconstituted with egg phosphatidylcholine

Lane 7, Normal major intrinsic protein reconstituted with dipalmitoylphosphatidylcholine

Lane 8, Normal major intrinsic protein reconstituted with egg phosphatidylcholine

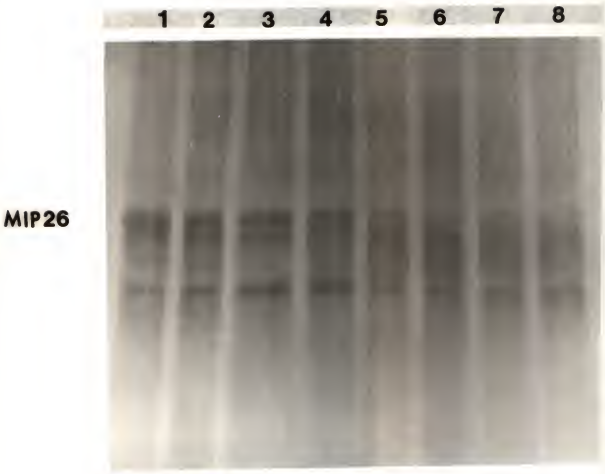

1 2 3 4 5 6 7 8
MIP26

Table 1: Protein and phospholipid recovery from reconstituted liposomes

| Initial protein (mg) | Initial total lipid extract (μmoles) | initial egg PC (μmoles) | initial DPPC (μmoles) | Protein recovered % | lipid recovered % |
|---|---|---|---|---|---|
| - | .113 | - | - | - | 35 |
| - | - | .113 | - | - | 34 |
| - | - | - | .113 | - | 34 |
| .260 | .113 | - | - | 81 | 95 |
| .260 | - | .113 | - | 97 | 93 |
| .260 | - | - | .113 | 84 | 95 |

Normal lens membrane protein were isolated by dissolving lens membranes in 1% sodium cholate in Tris/HCl buffer (pH=8.0) and dialyzing against 1% sodium cholate for 2 days. Total lipid extract was prepared by suspending the lens membranes in chloroform/methanol (2/1) and 0.88% KCl; after centrifugation and lower phase contained the total lipid extract.

Reconstituted liposomes containing cholate-solubilized protein and lipid or only lipid were prepared using cholate dialysis as described in Materials and Methods. Protein and lipid were assayed before and after reconstitution.

Figure 2 A: Fluorescence polarization of cis parinaric acid in:

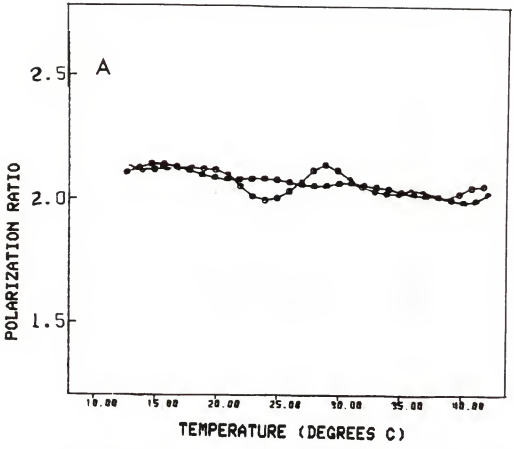
(O-O) membranes isolated from chick lenses, prepared as described in Chapter 1.

(●-●) reconstituted liposomes containing chick lens lipids and chick lens proteins, prepared as described in Materials and Methods

Figure 2 B: Fluorescence polarization of cis parinaric acid in:

(O-O) reconstituted liposomes containing egg phosphatidylcholine

(●-●) reconstituted liposomes containing chick lens membrane protein and egg phosphatidylcholine, prepared as described in Materials and Methods


A
2.5
2.0
1.5
POLARIZATION RATIO
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TEMPERATURE (DEGREES C)

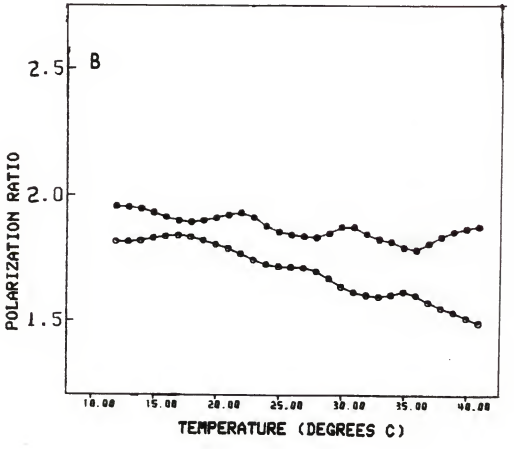

B
2.5
2.0
1.5
POLARIZATION RATIO
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TEMPERATURE (DEGREES C)

Figure 3: Fluorescence polarization of cis parinaric acid in:

(O-O) membranes isolated from human lenses

(●-●) reconstituted liposomes containing human lipid extract and human lens protein

(Δ-Δ) reconstituted liposomes containing human lipid extract. Liposomes were prepared as described in Material and Methods.

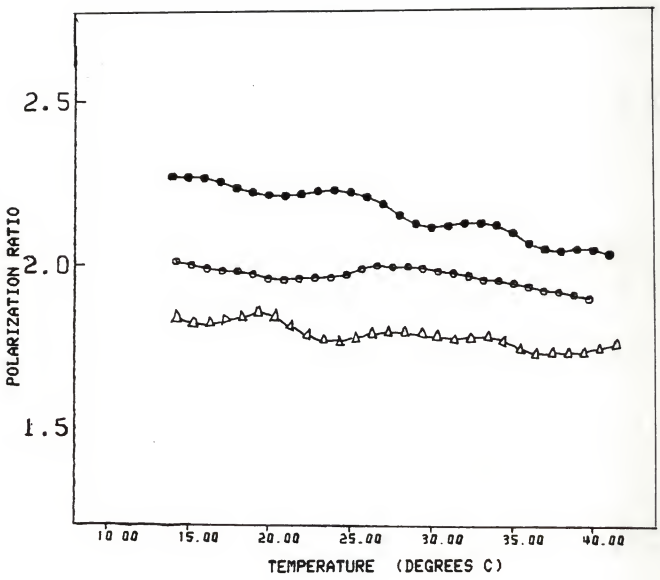

2.5
2.0
1.5
POLARIZATION RATIO
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TEMPERATURE (DEGREES C)

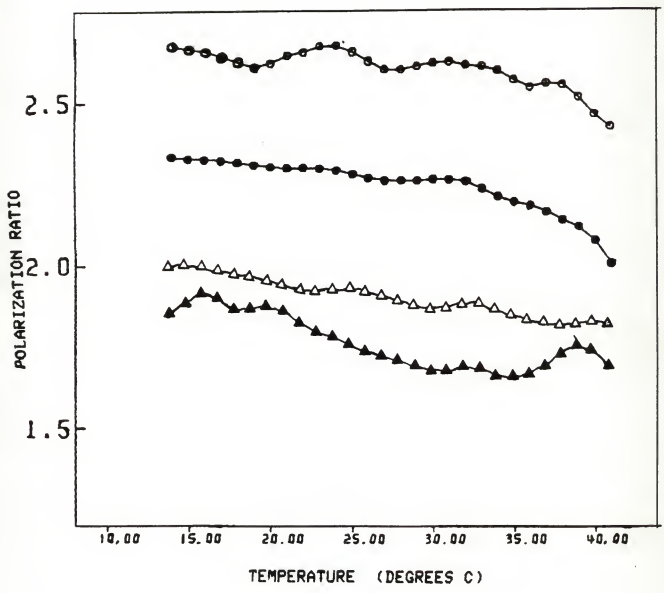
Figure 4: Fluorescence polarization of trans parinaric acid in:

(O-O) reconstituted liposomes containing human lens membrane protein and dipalmitoylphosphatidylcholine

(●-●) reconstituted liposomes containing dipalmitoylphosphatidylcholine

(Δ-Δ) reconstituted liposomes containing human lens membrane protein and egg phosphatidylcholine

(▲-▲) reconstituted liposomes containing egg phosphatidylcholine. All liposomes were prepared as described in Materials and Methods.


2.5
2.0
1.5
POLARIZATION RATIO
10.00
15.00
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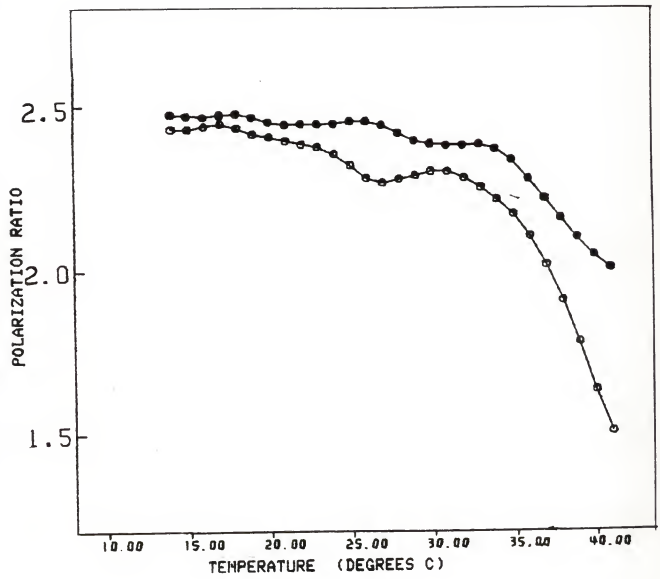
25.00
30.00
35.00
40.00
TEMPERATURE (DEGREES C)

Figure 5: Fluorescence polarization of *cis* parinaric acid in:

(●-●) reconstituted liposomes containing human lens membrane protein and dipalmitoylphosphatidylcholine

(0-0) reconstituted liposomes containing dipalmitoylphosphatidylcholine, prepared as described in Materials and Methods.


2.5
2.0
1.5
POLARIZATION RATIO
10.00
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20.00
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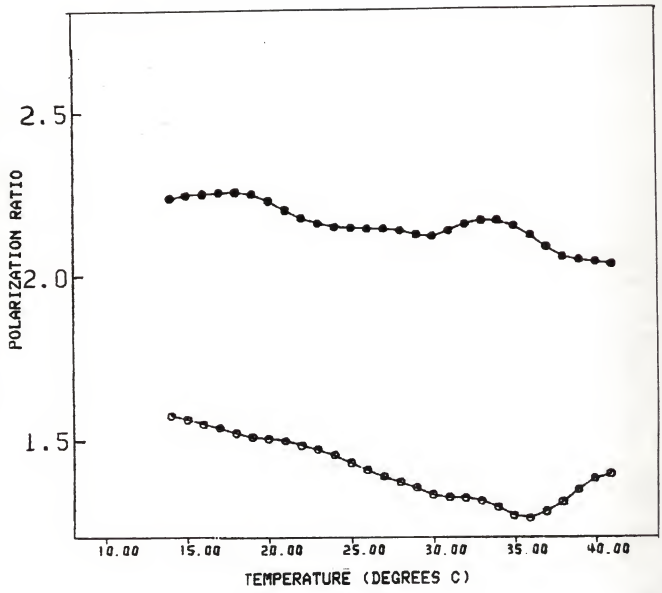
35.00
40.00
TEMPERATURE (DEGREES C)

Figure 6: Fluorescence polarization of cis parinaric acid in:

(●-●) reconstituted liposomes containing human lens membrane protein and egg phosphatidylcholine

(O-O) reconstituted liposomes containing egg phosphatidylcholine, prepared as described in Materials and Methods.


2.5
2.0
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POLARIZATION RATIO
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TEMPERATURE (DEGREES C)

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# MEMBRANE LIPID ORDER IN NORMAL AND CATARACTOUS HUMAN LENSES

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

It has been suggested by several investigators that disruption of the lens fiber cell member structure and/or function is an early event in the development of human senile cataract. It was therefore of interest to examine the motion and distribution of lens membrane lipids, using fluorescence depolarization techniques, in order to determine if these parameters are altered in cataractous lenses.

Membrane preparations from normal and cataractous human lenses were prepared by a procedure which minimally disrupts membrane lipid structure. Fluorescence depolarization of cis and trans-parinaric acid probes was measured as a function of temperature in membranes, lipid extracts, and phospholipids. These measurements indicated that membranes from cataractous lenses were less ordered (more fluid) than equivalent membranes from normal, age-matched control lenses. No significant differences could be detected between normal and cataractous membrane total lipid extracts, or between normal and cataractous membrane phospholipids. These observations imply that membrane lipid-protein interactions play a significant role in determination of lens membrane structure, and that these interactions are altered in cataractous lens membranes.

In order to study lipid/protein interactions, the main intrinsic protein was isolated from human lens membrane preparations using 1% sodium cholate. The protein was then reconstituted using a cholate-dialysis method with total lipid extract, egg PC and DPPC. Polarization ratios were calculated for reconstituted liposomes containing only lipid and reconstituted liposomes containing lipid and major intrinsic protein. Results indicate there is a stable association of the protein with the

lipid. The degree of lipid ordering depends on the lipid species used for reconstitution.